Reply to Perez and Patel

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We next exposed infected host cells to lysosomotropic alkalinizing agents to examine whether neutralizing lysosomal pH would reduce the formation of SCVs. Analysis of an average percentage of SCVs from 3 independent experiments revealed that host cells treated with alkalinizing agents exhibited lower percentages of SCVs 7 days after infection (Figure 1*E* and *F*). In 1 of 3 events, however, there was a peak in SCV percentage at day 5 for ammonium chloride–treated host cells infected with *S. aureus* 6850 and *S. epidermidis* IDRL-8933, resulting in a large mean standard deviation at that time point (Figure 1*F*). No differences were observed in total colony counts between cells that were and those that were not treated (Figure 1*D*).

In conclusion, low pH and the intracellular environment promote the formation of *S. epidermidis* SCVs. Acidic environments, such as within lysosomes or phagosomes, may induce SCV formation for *S. aureus* and *S. epidermidis*.

Notes

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Reply to Perez and Patel

To the Editor—We thank Perez and Patel for taking our recent observation on *Staphylococcus aureus* small-colony variants (SCVs) a step further [1]. We reported that low pH and intracellular localization promotes *S. aureus* SCV formation and that the frequency of SCVs is reducible by phagolysosomal alkalinization. Now Perez and Patel show that the same is true for select *Staphylococcus epidermidis* strains, including those derived from prosthetic joint infections [2].

The capability of *S. aureus* to reside and persist intracellularly is strain dependent, owing to differences in the presence and expression of virulence factors, and is also host-cell dependent [3]. This is most likely also true for *S. epidermidis*. The long-term *S. aureus* intracellular persistence model that we used in our study consisted of the human lung carcinoma cell line A549 and *S. aureus* strain Cowan. A549 cells are very robust and only exhibit limited sensitivity to *S. aureus*–induced cell damage [4]. *S. aureus* strain Cowan is highly invasive and does not express certain virulence factors, resulting in reduced host cell damage [5]. By exchanging the tissue culture medium daily, we maintained the antibiotic pressure constant over the course of infection. Discrepancy between the numbers of viable intracellularly persisting bacteria in the study by Perez and Patel and ours, thus, is most probably due to the use of another host cell line and another *S. aureus* strain. The *S. aureus* strain 6850 used as a control by Perez and Patel has been shown to modulate phagolysosomal pH, escapes phagolysosomes, and is cytotoxic [6].

In conclusion, low pH and intracellular localization promote formation of *S. aureus* and *S. epidermidis* SCVs. The frequency of these SCVs is reducible by chloroquine. These observations are most likely also applicable to other intracellular persisting bacterial infections.

Notes

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Codon 91 Gyrase A Testing Is Necessary and Sufficient to Predict Ciprofloxacin Susceptibility in *Neisseria gonorrhoeae*

To the Editor—We read with great interest the article by Grad et al [1]. We agree with their conclusion that gyrase A (*gyr*A) genotype testing of *Neisseria gonorrhoeae* is a valuable means of resistance testing; however, we believe that *gyr*A testing, specifically of codon 91, is both necessary and sufficient for predicting susceptibility to ciprofloxacin. There have been 11 studies (N=4777 specimens) comparing real-time polymerase chain reaction (RT-PCR) genotype results with conventional antimicrobial susceptibility testing methods, all of which have demonstrated high sensitivity and specificity (93.8%–100% and 93.2%–100%, respectively). Positive and negative predictive values were similarly impressive (94.4%–100% and 87.5%–100%, respectively). Furthermore, 4 studies found that mutation at codon 91 of the *gyr*A gene as determined by RT-PCR was 100% specific for *N. gonorrhoeae* compared with other *Neisseria* species [2–5].

Other mutations have been shown to contribute to ciprofloxacin resistance, but previous studies have shown that other mutations in general occur in conjunction with a mutation in the *gyr*A gene [6, 7]. In addition, it is estimated that approximately 80% of *N. gonorrhoeae* infections in the United States are susceptible to ciprofloxacin [8]. Those 2 facts support the implementation of *gyr*A genotype testing to promote the use of targeted ciprofloxacin therapy. That may in turn reduce overuse of ceftriaxone. A recent article showed that treatment may be a major driver of ceftriaxone resistance in *Neisseria gonorrhoeae* [9], which has been called one of the top 3 urgent threats to public health by the Centers for Disease Control and Prevention [10].

We developed a rapid codon 91 *gyr*A genotypic assay using RT-PCR techniques [6], and we verified the assay in accordance with Clinical Laboratory Improvement Amendments [2]. UCLA Health introduced that assay into routine clinical practice for all *N. gonorrhoeae*–positive specimens in November 2015. Further studies are underway to characterize the impact of that implementation.

Notes

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Reply to Allan-Blitz and Klausner

To the Editor—We thank Allan-Blitz and Klausner [1] for the citations to their group's work in this area and to the efforts underway to test diagnostics for quinolone resistance in *Neisseria gonorrhoeae*. Although our study investigated the genetic basis of resistance and assessed the positive and negative predictive values of specific mutations for resistance in the set of samples we analyzed [2], we take no position on the suitability of particular diagnostics. We note, however, that the US Food and Drug Administration has published guidance for antimicrobial susceptibility test systems [3]. The lower end of the range in negative predictive value cited by Allan-Blitz and Klausner (87.5%) is considerably lower than the 99% we observed,